

**REMARKS**

The Office Action of March 11, 2002, presents the examination of claims 1-9 and 11-18. Claim 9 is allowed. Claims 17 and 18 are amended to correct minor idiomatic errors. Specifically, the term "said" is added before the phrase "transcription control region" and the term "minimal" is amended to "minimum." A marked-up version of the claims showing the changes made is attached hereto. No new matter is inserted into the application.

***Claim Objections***

The Examiner objects to claims 17 and 18 for missing the word "said" in line 5. In response to the Examiner's remarks, Applicants amend claims 17 and 18 in accordance with the Examiner's suggestions. Thus, the instant objection is overcome.

***Rejection under 35 U.S.C. § 112, second paragraph***

The Examiner rejects claim 18 under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite. Applicants respectfully traverse. Reconsideration of the claim and withdrawal of the instant rejection are respectfully requested.

Specifically, the Examiner asserts that it is unclear whether "at least one inert nucleotide" is a part of the promoter or the recognition sequence of the transcription control rejection. In response to the Examiner's remarks, Applicants amend claim 18 to clarify that "at least one inert nucleotide" is a part of the transcription control rejection.

Applicants respectfully submit that the instant claims are in full compliance with 35 U.S.C. § 112, second paragraph, such that the instant rejection is overcome.

***Rejection under 35 U.S.C. § 102***

The Examiner maintains the rejection of claims 1-3, 5-8, 11-15, and 17 under 35 U.S.C. § 102(b) for allegedly being anticipated by Evans '429 (USP 5,298,429). Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Applicants respectfully submit that Evans '429 fails to anticipate the cell recited in claim 1. Evans '429 in example II.F.(d) teaches that pMTVCAT is introduced into a CV-1 cell. It is emphasized that such teachings in Evans '429 fail to describe that in the test cell thereof, the selective marker is in a molecule with the reporter gene. Evans '429, in describing

the test cell, fails to describe the conditions in which the selective marker and reporter gene are present therein. It follows that example II.F.(d) fails to describe the cell recited in claim 1.

Evans '429 describes in Fig. II-1 that two plasmids are introduced into a cell. However, this is merely a general description. Fig. II-1 does not describe that the two plasmids are introduced into the cell in any particular way. It follows therefore, that Fig. II-1 fails to describe whether the cell resulting from the introduction of the plasmids is actually a cell which stably maintains the reporter gene and the selective marker. Fig. II-1 encompasses transient introductions of said two plasmids, but does not encompass stable maintenance of two plasmids in a cell. As such, Fig. II-1 fails to describe the cell recited in claim 1.

Further, Applicants respectfully submit that the AMP gene described in Fig. II-1 is very different from a selective marker gene which can function in an animal cell, as recited in the instant claims. This is because the AMP gene does not function as a selective marker gene in an animal cell. The attached "Molecular Cloning, volume 1", page 1.148 by Sambrook and Russell evidences that the AMP gene would not function as a

selectable marker in an animal cell. For example, page 1.148 teaches that the lethal activity of ampicillin involves inhibiting the production of certain glycan chains present in a cell wall. There are, of course, no cell walls present in an animal cell. Such properties of the animal cell evidence that the utilization of the AMP gene in an animal cell would provide no selectivity, because ampicillin would be unsuccessful in presenting the lethal activity on the animal cell. It is the resistance to the lethal activity of ampicillin, which allows the AMP gene to function as a selectable marker. As there is no lethal activity in connection with an animal cell, the AMP gene would not function as a selectable marker. An animal cell would survive in the presence of ampicillin with or without the AMP gene present therein. It follows that the AMP gene in an animal cell would fail to selectively mark any animal cell in which the AMP gene is present therein.

Fig. II-1 also describes that one of the plasmids thereof encodes a hGR gene and the other plasmid thereof encodes a CAT gene. The hGR gene is a species of the hormone receptor gene thereof. The CAT gene is a species of the reporter gene thereof. Evans '429 describes that the hormone receptor gene and the reporter gene are distinct from the selective marker

gene thereof. In this regard, Fig. II-1 fails to describe that in the test cell thereof, the reporter gene is in a molecule with a selective marker which is functional in an animal cell.

For the above reasons, Applicants respectfully submit that Evans '429 fails to anticipate the present invention. Withdrawal of the instant rejection is respectfully requested.

### **Conclusion**

Applicants respectfully submit that the above remarks and/or amendments fully address and overcome the outstanding rejections and objections. For the foregoing reasons, Applicants respectfully request the Examiner to withdraw all of the outstanding rejections and objections, and to issue a notice of allowance indicating the patentability of the present claims. Early and favorable action of the merits of the present application is thereby respectfully requested.

If there are any minor matters precluding allowance of the application which may be resolved by a telephone discussion, the Examiner is respectfully requested to contact Kristi L. Rupert, Ph.D. (Reg. No. 45,702) at (703) 205-8000.

If necessary, the Commissioner is hereby authorized in this, concurrent, and further replies, to charge payment or


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credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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GMM/KLR:gml  
2185-0424P

Attachments: Version with Markings to Show Changes Made  
Sambrook and Russell, Molecular Cloning, p. 1.148

MARKED UP VERSION SHOWING CHANGES MADE

IN THE CLAIMS

The following claims are amended:

17. (Amended) An animal cell expressing a gene coding a ligand-responsive transcription control factor and securely maintaining a DNA comprising in a molecule, the following genes (a) and (b):

(a) a reporter gene connected downstream from a transcription control region; wherein said transcription control region contains a [minimal] minimum promoter and a recognition sequence of the ligand-responsive transcription control factor and contains no sequence having the transcription control ability substantially changed by the ligand-responsive transcription control factor recognition sequence and minimum promoter; and

(b) a selective marker gene which can function in said cell;

and provided that the following gene (c):

(c) a reporter gene connected downstream from a promoter which transcription activity is unchanged by having said responsive transcription control factor contacted

with a ligand of said ligand-responsive transcription control factor, said reporter gene (c) coding a protein which can be differentiated from the protein coded by said gene (a)

is not present in said cell.

18. (Amended) An animal cell expressing a gene coding a ligand-responsive transcription control factor and securely maintaining a DNA comprising in a molecule, the following genes (a) and (b):

(a) a reporter gene connected downstream from a transcription control region; wherein said transcription control region consists of a [minimal] minimum promoter, at least one inert nucleotide, and at least one recognition sequence of the ligand-responsive transcription control factor [and at least one inert nucleotide]; and

(b) a selective marker gene which can function in said cell;

and provided that the following gene (c):

(c) a reporter gene connected downstream from a promoter which transcription activity is unchanged by having



said responsive transcription control factor contacted with a ligand of said ligand-responsive transcription control factor, said reporter gene (c) coding a protein which can be differentiated from the protein coded by said gene (a)

is not present in said cell.

VOLUME

1

# Molecular Cloning

A LABORATORY MANUAL

THIRD EDITION



[www.MolecularCloning.com](http://www.MolecularCloning.com)

Sambrook and Russell



## 1.148 Chapter 1: Plasmids and Their Usefulness in Molecular Cloning

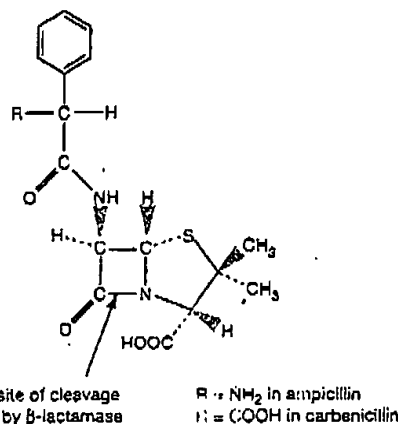
## AMPICILLIN AND CARBENICILLIN

## Properties and Mode of Action of Ampicillin and Carbenicillin

- Ampicillin is an aminopenicillin; carbenicillin is a semi-synthetic carboxypenicillin (please see Figure 1-16).
- The antimicrobial activity of both compounds extends to Gram-negative organisms such as *Haemophilus influenzae* and *E. coli*. Carbenicillin was the first penicillin with significant activity against *Pseudomonas* species that are not susceptible to aminopenicillins, such as ampicillin.
- The rigidity of the cell walls of susceptible organisms is due in part to a thin layer of cross-linked peptidoglycan, just 1–2 molecules thick. The long glycan chains are composed entirely of amino sugars and are cross-linked by peptide chains containing D-amino acids. The penicillins inhibit the final stage of synthesis of cross-links, which occurs outside the cell and is catalyzed by a transpeptidase (for reviews, please see Neu 1985; Donowitz and Mandell 1988). Penicillins are most active against bacteria in the logarithmic phase of growth and have relatively little effect in the stationary phase, when synthesis of peptidoglycan is suppressed.
- In addition to their activity against transpeptidase, penicillins inhibit enzymes (called penicillin-binding proteins or PBPs) necessary for the rod-like structure of *E. coli* and for septum formation during division (Tomasz 1986).

## Mechanism of Resistance to Ampicillin and Carbenicillin

- The periplasmic enzyme  $\beta$ -lactamase catalyzes hydrolysis of the cyclic amide bond of the  $\beta$ -lactam ring, with concomitant detoxification of ampicillin and carbenicillin (Abraham and Chain 1940; Bush and Sykes 1984).
- $\beta$ -lactamases are present in small amounts in the periplasmic space of field strains of antibiotic-resistant, Gram-negative bacteria. They are coded either chromosomally or on plasmids. The most prevalent  $\beta$ -lactamase in Gram-negative bacteria — the TEM  $\beta$ -lactamase — is named after the initials of the Athenian girl from whom a strain of *E. coli* expressing the enzyme was first isolated in 1965 (Datta and Kontomichalou 1965). The TEM  $\beta$ -lactamase, which is widely used as a selectable marker in molecular cloning, is a 286-residue protein encoded by the *bla* gene (Sutcliffe 1978). The first 23 amino acids of the nascent lactamase protein function as a signal sequence cleaved during translocation of the protein into the periplasm.
- When  $\beta$ -lactamase is expressed from high-copy vectors, such as those used in molecular cloning, large amounts of the enzyme are secreted into the medium. Sufficient  $\beta$ -lactamase can be produced by a single transformed colony to hydrolyze the antibiotic in the surrounding medium and to create a protected zone in which antibiotic-sensitive colonies can grow. This leads to the appearance of nontransformed, satellite colonies. The problem is ameliorated, but not completely eliminated, by using carbenicillin rather than ampicillin in selective media, because carbenicillin is more resistant than ampicillin to hydrolysis by the  $\beta$ -lactamases of genera such as *Pseudomonas* and *Escherichia*.

FIGURE 1-16 Cleavage of  $\beta$ -Lactam Antibiotics by  $\beta$ -Lactamase

Chemical bonds (filled arrows) appear above the plane of the figure; bonds (dashed lines) appear below the plane of the figure.